

RECOVERY OF PROTEINS FROM LIME-SULFIDE EFFLUENTS FROM UNHAIRING CATTLEHIDES*

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ABSTRACT

Proteins of 90 to 92 percent purity that were odorless and slightly acidic in taste, and varied from white to cream color were recovered from the lime-sulfide hair-pulping effluents of five tanneries.

Proteins were recovered and purified as follows: suspended solids were removed by gravity sedimentation or screening, by centrifugation or filtration, or by both processes; soluble inorganic compounds (sodium sulfide, calcium hydroxide, etc.) were removed by dialysis or ultrafiltration; proteins were precipitated by acidification, and then washed and dried.

Yields of protein product recovered from the undiluted lime-sulfide effluents were: paddle vats—one lb., from 18 to 20 gal.; hide processors—one lb., from seven to nine gal.

Protein content, amino acid composition, and yield of the protein fraction precipitated at pH 5.0 differed from those of the protein fraction precipitated at pH 3.8.

During storage of the lime-sulfide effluent at room temperature, continued hydrolysis changed the composition of the effluent and decreased the yield of protein.

Amino acid composition of the protein was close to that of native hair. It contained the ten essential amino acids but was lower in most than whole egg protein and would need supplementation for use as feed.

Recovery of the proteins by the procedures described would lower the chemical oxygen demand and biochemical oxygen demand of the effluent, allow for possible recycling of chemicals, and produce a potentially useful product.

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INTRODUCTION

Removal of hair from cattlehides by the lime-sulfide hair-pulping process adds substantial amounts of solubilized protein to the effluent and increases its chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Removal of the pollutants is difficult and expensive.

Feairheller *et al.* (1) recovered and analyzed hair proteins from solutions in which hair, clipped from the hide, was treated with sodium sulfide and calcium hydroxide under conditions approximating those used in a tannery for pulping hair. Under these conditions about 75 percent of the hair was dissolved and about 85 percent of the dissolved hair protein could be recovered as a white or tan precipitate.

This paper reports the extension of that research to the recovery of proteins from the lime-sulfide unhairing effluents of several tanneries, and the analyses and nutritional value of the recovered proteins.

EXPERIMENTAL

Samples of lime-sulfide unhairing effluents were obtained from five side-leather tanners who use the high-sulfide, hair-pulping process. The concentrated effluents were obtained directly from the unhairing operations and did not include any wash water. The raw stock used included brine-cured, green-salted, and fresh hides, which were unhaired in hide processors or in drums or paddle vats. Examination showed that the effluents were complex mixtures of materials present as suspended insoluble solids, some in colloidal and some in true solution. The insoluble solids were mainly partially disintegrated hair, pieces of fat, particles of hide, and lime. Proteins and possibly some emulsified fat and sulfur were in colloidal solution. Some of the proteins and the inorganic compounds, including salt, lime, and sulfides, were in true solution.

The composition of lime-sulfide effluent from a tannery using fresh hides and a paddle was determined.

Recovery and Purification of Proteins

High purity proteins were recovered from lime-sulfide hair-pulping effluents by the following four-step procedure:

1. Separation of suspended solids from solution.
2. Separation of soluble inorganic compounds.
3. Acid precipitation of the proteins.
4. Washing and drying of the proteins.

1. Separation of Suspended Solids from Solution

Because of its complexity, clarification of the lime-sulfide effluent is difficult. Most of the larger suspended solids can be removed by gravity sedimentation or

by screening through 20-, 40-, and 65-mesh screens. The effluent could be completely clarified with filter paper, but the filtration rate was very slow. Addition of Celite Filter Aid‡ did not increase the filtration rate or improve clarification.

All suspended solids except fat could be removed quickly and effectively by two-stage centrifugation. A ten-min. centrifugation in an International centrifuge at 2,100 r.p.m. (988 gravity) removed most of the suspended solids, which formed a cake on the bottom of the tube. Most of the fat, which floated on the surface, could be removed by skimming, then filtering through glass wool or a bed of coarse sand. The effluent then was clarified by centrifugation in a Sharples Super centrifuge at 25,000 r.p.m. with a continuous feed, or in a Beckman Ultra-centrifuge at 4,000 r.p.m. in a batch operation. The black melanin pigment, which was one of the last of the suspended solids to be removed, required 2,000 gravity for ten min.

2. Separation of Soluble Inorganic Compounds

Dialysis. To separate salt, lime, and sulfides, the clarified protein solutions were dialyzed exhaustively with running water, until a test for sulfides with lead acetate paper was negative. The regenerated cellulose tubing used in these studies retained all substances with molecular weights of 12,000 and higher.

Ultrafiltration. Another portion of the clarified protein solution was purified by ultrafiltration, which is similar to dialysis, except for the fact that the protein solution is under pressures up to 100 p.s.i. The protein solution, contained above the membrane and agitated by a magnetic stirrer, was washed by the continuous addition of water under pressure until it was free of inorganic compounds.

Ultrafiltration is faster and more efficient than dialysis and requires less water, and the solutions can be concentrated, without heating, to 25 percent or more protein solids.

3. Acid Precipitation of the Proteins

During dialysis or ultrafiltration, as the pH dropped from 12.8 to near neutrality, the protein solution became milky and a small amount of material became insoluble and settled to the bottom of the tube; its identity is being investigated.

The protein solutions, purified by dialysis or by ultrafiltration, were slowly acidified to pH 4.2 with glacial acetic acid. Protein began to precipitate at about pH 6.0 and continued to precipitate down to about pH 3.8; most precipitated between 4 and 5. At pH 4.2 the protein solution appeared to act as a buffer. Acid did not precipitate the protein completely and small amounts remained in the supernatant solution.

Yields of proteins from lime-sulfide effluents were reported to be highest at pH 4.0 by Blazej *et al.* (2) in Czechoslovakia, and at pH 4.3 by Niwa (3) in

‡Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

Japan. Jones and Mecham (4) obtained maximum precipitation of the keratin from wool, feathers, hoof, and hog hair at pH 4.2.

The proteins also can be precipitated by other acids and by precipitating agents. For example, ferripolyphosphate produced a voluminous white precipitate, an iron polyphosphate-protein complex.

4. *Washing and Drying of the Proteins*

The acid-precipitated proteins were allowed to settle overnight in the refrigerator. After the supernatant solutions were decanted, the proteins were washed several times by resuspending in distilled water, centrifuging, and decanting. The proteins then were freeze-dried.

The various steps in the recovery and purification of the proteins are illustrated in Figure 1.

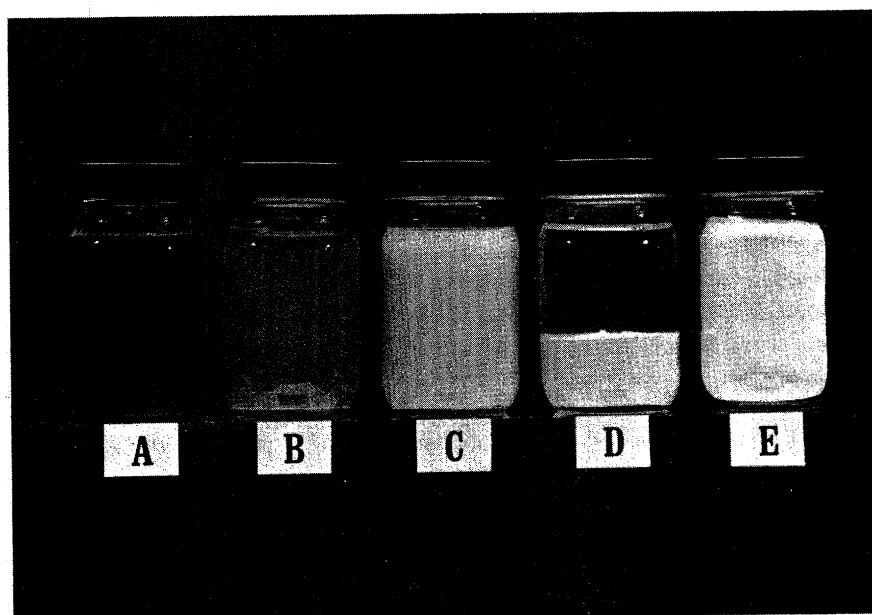


FIGURE 1.—Illustration of the lime-sulfide effluent and the steps in the protein recovery procedure: "A," undiluted effluent; "B," effluent clarified by centrifugation; "C," effluent after dialysis to neutrality; "D," protein after acid precipitation; "E," freeze-dried product.

Preparation of Proteins Precipitated at Different pH's

To determine the effect of the precipitation pH on the composition of the proteins, a sample of effluent from cured hides unhaired in a paddle was clarified by the two-stage centrifugation described previously, then dialyzed until sulfide-free. Glacial acetic acid was added slowly to pH 5.0. The precipitated protein was washed several times by centrifuging and decanting as described above. The

supernatant solution and washings then were acidified further to pH 3.8 with glacial acetic acid and the precipitated protein was washed as above. The recovered proteins then were freeze-dried.

Effect of Aging of Effluent on Protein Recovery and Composition

To determine the effect of aging of the effluent on protein recovery and composition, a two-gal. sample of concentrated lime-sulfide effluent from the unhairing of cured hides in a hide processor was divided into three equal volumes. The control portion was processed immediately and the other two portions were stored for 30 days, one at 2°C. and one at 25°C.

Nutritional Value of Proteins

The nutritional values of the recovered proteins were determined by the Pharmacology Laboratory at the United States Department of Agriculture's Western Regional Research Center.

ANALYTICAL METHODS

Total ash and fat were determined by the Official Methods of Analysis of the ALCA (5). Nitrogen was determined by the semi-micro Kjeldahl method. Total organic and inorganic sulfur were determined by micro methods. Sodium sulfide was determined by the Official Method of Analysis of the Society of Leather Trades' Chemists (6).

The samples of protein were prepared for amino acid analysis by hydrolyzing in 6 N hydrochloric acid solution for 24 hrs. under an atmosphere of nitrogen. Excess hydrogen chloride was removed by repeated evaporations under reduced pressure with intermittent additions of distilled water. The final residues then were diluted to a known volume with 0.1 N hydrochloric acid. Analyses were run in triplicate on a Piez-Morris-type ion-exchange column with a continuous, gradient-elution buffer (7). Tryptophan was determined on a separate sample by the standard chromatographic technique on an alkaline hydrolysate.

The official methods of analysis of the American Public Health Association, American Water Works Association, and the Water Pollution Control Federation were used for pH, BOD, COD, total solids, volatile solids, suspended solids, and volatile suspended solids (8).

The Official Method of Analysis of the AOAC was used to determine the protein efficiency ratio (PER) (9).

RESULTS AND DISCUSSION

Composition of Lime-Sulfide Effluent

The composition of lime-sulfide effluent without any wash water from a tannery using fresh hides and a paddle is given in Table I. The BOD was 19,125 p.p.m. Calculated to a daily volume of 20,000 gal., the total load from the un-

TABLE I
COMPOSITION OF LIME-SULFIDE UNHAIRING EFFLUENT*

	p.p.m.		
BOD, 5 days 20°C.	19,125	pH	12.8
COD	36,639	Na ₂ S, %	0.55
Total Solids	56,900	Total N, %	0.41
Total Volatile Solids	36,160	Floating Solids†, ml./L.	100
Suspended Solids	25,450	Settleable Solids†, ml./L.	300
Volatile Suspended Solids	16,790		

*Fresh hides, paddle vat, no wash water.

†One hr. at 25°C.

diluted unhairing effluent would be 3,190 lbs. BOD per day. The COD value was about twice that of the BOD. Approximately 64 percent of the total solids were organic and volatile at 600°C. About half of the total solids were suspended and 66 percent of the suspended solids were volatile. The calculated value of 31,450 p.p.m. solids in solution was obtained by subtraction of the suspended solids from the total solids.

The effluent, pH 12.8, contained 0.55 percent Na₂S and 0.41 percent total nitrogen. The nitrogen present would be equivalent to about 2.5 percent protein, using a factor of 6.25. In one hr. in the Imhoff cone, 100 ml. of solids per liter, mainly fat and undissolved hair, floated to the surface and 300 ml. of solids per liter settled.

Separation of Na₂S by Ultrafiltration

Table II shows the purification of a clarified lime-sulfide effluent by ultrafiltration using a membrane that retains substances with molecular weights of

TABLE II
PURIFICATION OF A CLARIFIED LIME-SULFIDE EFFLUENT
BY ULTRAFILTRATION

	Vol. (ml.)	Na ₂ S	
		Concentration (%)	Fraction of Original (%)
Clarified Effluent	350	0.58	—
Ultrafiltrate			
1	350	0.49	84.5
2	350	0.08	13.8
3	158	0.004	0.7
			99.0
Purified Effluent	305	0.006	1.0

10,000 or higher. Effluent from fresh hides, unhaired in a paddle, was clarified by the two-stage centrifugation described previously, then concentrated from 350 ml. to 75 ml. by ultrafiltration at 60 p.s.i. The effluent then was diluted to 350 ml. with distilled water. Ultrafiltration was continued at 60 p.s.i., maintaining constant volume by continuous addition of distilled water. Eighty-five percent of the Na_2S was separated from the protein solution in the first equal volume of ultrafiltrate. This first ultrafiltrate could possibly be reused for unhairing by the addition of more Na_2S and lime. By ultrafiltration of the effluent with distilled water equal to about 2.4 times its volume, 99 percent of the Na_2S originally present was separated and transferred to the ultrafiltrate. The concentration of Na_2S in the purified effluent was reduced to 0.006 percent, one percent of the original amount.

COD and BOD Reduction by Protein Recovery

In an experiment to determine the reduction of the COD and the BOD by recovery and removal of the protein, another sample of effluent from fresh hides, unhaired in a paddle, was clarified by the two-stage centrifugation described above. The solid material separated by centrifugation accounted for approximately 30 percent of the COD and 37 percent of the BOD, as shown in Table III. Centrifugation in the International centrifuge accounted for 8.8 percent of the COD and 25.3 percent of the BOD. Centrifugation in the Beckman ultracentrifuge accounted for 20.9 percent of the COD and 11.9 percent of the BOD. The ultrafiltrate, four times the volume of the effluent, contained most of the sulfide and accounted for 31.5 percent of the COD and 34.9 percent of the BOD. By recovery and removal of the protein, the COD and BOD would be reduced by approximately 38 percent and 28 percent, respectively.

Yields of Proteins

The following yields of protein product were recovered from the undiluted lime-sulfide effluents: paddle vats—one lb., from 18 to 20 gal.; hide processors—one lb., from 7 to 9 gal.

Analyses of Recovered Proteins

The analyses of proteins recovered from the concentrated, lime-sulfide hair-pulping effluents of three side-leather tanneries are shown in Table IV. One tanner used fresh hides and a paddle; two tanners used cured hides and hide processors. The protein products were odorless and slightly acidic in taste, and varied from white to cream color. All three samples were similar in composition. The ash content was low, varying from 0.1 to 0.4 percent. The product from the fresh hides contained three percent fat, approximately ten times more fat than the products from the cured hides. The total sulfur values given here were 0.6 to 1.5 percent higher than the protein sulfur calculated from the amino acid analyses. Presumably this difference was free sulfur. The total (Kjeldahl)

TABLE III
CHANGES IN THE COD AND BOD PRODUCED BY THE PROTEIN RECOVERY PROCEDURES

	COD (p.p.m.)	Change in COD		BOD (p.p.m.)	Change in BOD	
		(p.p.m.)	(% Total)		(p.p.m.)	(% Total)
Lime-Sulfide Effluent	47,000	—	—	29,700	—	—
After International Centrifuge	42,854	4,146	8.8	22,200	7,500	25.3
After Ultracentrifuge	33,024	9,830	20.9	18,650	3,550	11.9
After Ultrafiltration	17,856	15,168	32.3	8,283	10,367	34.9
		29,144	62.0		21,417	72.1
Ultrafiltrate	14,783	—	31.5	10,367	—	34.9
Protein Solution	17,856*	—	38.0*	8,283*	—	27.9*

*The COD and BOD represented by the protein.

TABLE IV
ANALYSES OF RECOVERED PROTEINS*

Raw Stock	Unhairing Equipment	Moisture (%)	Ash (%)	Fat (%)	Total S (%)	Protein S (%)†	Total N (%)	Protein (%)†
Fresh	Paddle	4.1	0.1	3.0	3.8	3.2	15.5	89.7
Cured	Processor	3.3	0.4	0.4	4.2	3.3	15.8	92.1
Cured	Processor	3.6	0.1	0.3	5.7	4.2	16.1	91.2

*Moisture-free basis.

†Calculated from the amino acid analyses.

nitrogen contents were about one percent higher than the theoretical values calculated from the amino acid analyses. These latter values did not include amide nitrogen, which could conceivably account for the difference. The samples contained 90 to 92 percent protein, calculated from the amino acid analyses.

Amino Acid Composition of Recovered Proteins

The amino acid composition of the recovered proteins is given in Table V. Concentrations are given as percent by weight. The amino acid content of the

TABLE V
AMINO ACID COMPOSITION OF RECOVERED PROTEINS*

Amino Acid	Fresh Paddle	Cured Processor	Cured Processor	Native Hair	Whole Egg
<i>Essential</i>					
Arg	8.5 ± 0	9.25 ± 0.15	8.8 ± 0.2	9.7	6.1
His	1.0 ± 0.1	1.0 ± 0	0.9 ± 0.1	1.0	2.4
Ile	3.45 ± 0.05	3.4 ± 0.1	2.95 ± 0.05	3.4	6.6
Leu	7.0 ± 0	7.2 ± 0	5.6 ± 0.2	7.1	8.8
Lys	3.4 ± 0	3.2 ± 0	2.3 ± 0.1	3.4	6.4
Met	0.6 ± 0	0.5 ± 0	0.3 ± 0	0.4	3.1
Cys†	7.45 ± 0.15	7.45 ± 0.15	9.7 ± 0.1	12.4	2.3
Phe	2.35 ± 0.05	2.2 ± 0	1.95 ± 0.05	2.6	5.8
Tyr‡	3.9 ± 0.1	3.7 ± 0	3.4 ± 0.2	3.7	4.3
Thr	5.4 ± 0.1	5.5 ± 0.1	6.3 ± 0	6.1	4.9
Trp**	0.4	0.4	0.5	0.5	1.6
Val	4.5 ± 0.1	4.7 ± 0.1	4.5 ± 0.1	5.2	7.4
<i>Nonessential</i>					
Ala	3.1 ± 0.1	3.1 ± 0.1	2.6 ± 0.1	3.6	5.9
Asp	6.85 ± 0.15	6.6 ± 0.1	4.9 ± 0.2	6.6	9.6
Glu	14.15 ± 0.15	14.8 ± 0.2	12.95 ± 0.25	15.1	12.7
Gly	2.75 ± 0.15	2.6 ± 0	2.8 ± 0.1	4.3	3.3
Pro	5.25 ± 0.25	5.4 ± 0.2	6.8 ± 0	7.1	4.2
Ser	5.75 ± 0.15	6.0 ± 0	6.95 ± 0.15	8.2	7.6
<i>Uncommon</i>					
Lan††	3.85 ± 0.25	5.35 ± 0.15	6.5 ± 0.1	0	—
Lya‡‡	0.2 ± 0	0.2 ± 0	0.2 ± 0	0	—
Unknown***	0.3 ± 0	0.5 ± 0	0.75 ± 0.05	0	—

*Concentrations are weight percent; actual experimental range found in three separate runs.

†Sparing amino acid for methionine.

‡Sparing amino acid for phenylalanine.

**Run only once on a separate sample.

††Lanthionine; may have sparing effect on methionine.

‡‡Lysinoalanine.

***Unknown, elutes between methionine and isoleucine. Calculated using the leucine color constant.

proteins is close to that of native hair. The proteins from two tanneries showed considerable decrease in cystine, which was partially destroyed during the unhairing process. In all other respects the proteins from the three tanneries were remarkably uniform in composition. All eight amino acids essential for human nutrition, as well as histidine and arginine, were present. In addition to the eight essential amino acids, rats require histidine and chicks require both histidine and arginine. Compared with whole egg, the hair protein was low in histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, and valine. Cystine and tyrosine are listed with the essential amino acids because they may have a sparing effect on methionine and phenylalanine, respectively. That is, nutritionally, cystine could substitute for some of the methionine and tyrosine could substitute for some of the phenylalanine.

In addition to cystine and tyrosine, eight other nonessential amino acids were present, including lanthionine, formed during unhairing, and lysinoalanine, which Fearheller *et al.* (10) found was also formed during unhairing. Lanthionine may possibly also have a sparing effect on methionine. The protein also contained an unknown amino acid, eluting between methionine and isoleucine, as reported by Fearheller *et al.* (1).

Composition of Proteins Precipitated at Different pH's

Table VI shows partial amino acid compositions, on a percent by weight basis, of the proteins precipitated at pH 5.0 and at pH 3.8. Only amino acids that had substantial differences in concentration are shown. As would be expected, the more highly crosslinked and less acidic portion of the protein precipitated first at pH 5.0, then the less highly crosslinked and more acidic portion precipitated at pH 3.8. The protein contents of the products precipitated at pH's 5.0 and 3.8 were 91.2 percent and 93.3 percent, respectively, calculated from the amino acid analyses. Approximately 76 percent of the protein precipitable by acid precipitated at pH 5.0 and 24 percent at pH 3.8.

Effect of Aging of Effluent on Composition of Recovered Proteins

Analyses of the proteins recovered from effluents stored for 30 days at 2°C. and at 25°C. are given in Table VII. The product from the effluent stored at 2°C. was similar in composition to that of the control, except that a 17 percent better yield was obtained and the color was gray-white instead of white. Total sulfur and free sulfur (total sulfur minus protein sulfur) also increased.

The product from the effluent stored at 25°C. differed considerably from that from the control. Total sulfur and protein sulfur decreased to about half and two thirds, respectively, of their original concentrations. The protein content, calculated from the amino acid analyses, also decreased from 95 percent to 87 percent. The most pronounced change was the decrease of protein product re-

TABLE VI

EFFECTS OF PROTEIN PRECIPITATION pH AND OF AGING OF EFFLUENT
ON AMINO ACID COMPOSITION OF RECOVERED PROTEINS

Amino Acid*	Precipitation pH		Effluent Processed		
	5.0	3.8	Immediately	Aged 30 days at	
				2°C.	25°C.
Ala	3.0 ±0	3.85±0.05	—	—	—
Asp	6.1 ±0.1	9.4 ±0.3	6.4 ±0	7.05±0.15	7.85±0.15
Cys	—	—	5.4 ±0	4.75±0.05	0.55±0.05
Glu	15.0±0.1	20.75±0.75	14.6 ±0	15.8 ±0.2	16.25±0.25
Lan	15.2 ±0.2	7.1 ±0.3	9.0 ±0	7.45±0.05	10.1 ±0
Leu	6.9 ±0	10.2 ±0.2	6.55±0.15	7.8 ±0.1	8.9 ±0.1
Lys	2.7 ±0	3.8 ±0	2.8 ±0	3.3 ±0	2.3 ±0.1
Pro	5.7 ±0	3.05±0.15	6.15±0.05	5.25±0.25	4.35±0.15
Ser	4.8 ±0.1	3.85±0.05	6.45±0.05	5.05±0.05	2.8 ±0.1
Thr	5.1 ±0.1	4.3 ±0.2	5.9 ±0	4.9 ±0.1	3.3 ±0.1
Unknown	—	—	0.8 ±0	0.6 ±0.1	0.2 ±0
Nitrogen, %	16.5	15.8			
Protein, %†	91.2	93.3			
Yield, g.‡	4.1	1.3			

*Concentrations are weight percent; actual experimental range found in three separate runs.

†Calculated from the amino acid analyses.

‡Product recovered from 400 ml. of clarified effluent.

covered to only 47 percent of that of the control. Also, the recovered protein was beige in color and had a gummy consistency.

Portions of the amino acid analyses of the proteins recovered from effluents stored for 30 days at 2°C. and at 25°C. are shown in Table VI. Only those amino acids that had substantial differences in concentration at the 25°C. storage are given. The composition of protein from the effluent stored at 2°C. was close to that of the control. However, the protein from the effluent stored at 25°C. was considerably different from that of the control. The sulfur-containing amino acids decreased considerably. Cystine, for example, decreased to one tenth of its original value and serine and threonine decreased markedly. Aspartic acid, glutamic acid, lanthionine, and leucine increased in concentration.

These results indicate that storage of the effluent for 30 days at 25°C. produces undesirable changes in the yield, properties, and composition of the protein product.

TABLE VII

EFFECT OF AGING OF EFFLUENT ON COMPOSITION AND QUANTITY OF RECOVERED PROTEINS*

	Moisture (%)	Ash (%)	Fat (%)	Total S (%)	Protein S (%)†	N (%)	Protein (%)†	Protein Recovery (g./gal.)	Color
Control	8.8	0.1	0.3	4.6	3.3	16.2	94.6	64	white
30 days 2°C.	3.9	0.1	0.1	5.0	2.9	16.2	92.5	75	gray-white
30 days 25°C.	1.6	0.2	0.3	2.2	2.1	15.7	87.1	30	beige

*Moisture-free basis.

†Calculated from the amino acid analyses.

Nutritional Value of the Recovered Proteins

One objective of our present study was to determine the nutritional quality of the recovered proteins. The results of an abbreviated nutritional test are given in Table VIII. Five rats were used in each group. The control group was fed ten percent casein. The test group was fed a mixture of ten percent soy protein and five percent hair protein fortified with 0.1 percent lysine. Another group

TABLE VIII
NUTRITIONAL VALUE OF PROTEINS RECOVERED FROM
TANNERY EFFLUENT

Dietary Source of Protein	Final Mean Body Wt. of Rats* ± std. dev. (g.)	PER†	Nitrogen Digestibility‡ (%)
10% casein control	162 ± 16	2.50	93
15% soy protein	133 ± 11	2.30	83
10.0% soy protein 5.0% hair protein 0.1% lysine	95 ± 11	1.69	74

*After 14 days, five rats per group; initial weight 53 g.

†Protein efficiency ratio is the weight gain divided by the grams of protein intake; the PER values given are the actual values which have been corrected to 2.50 for casein.

‡% nitrogen digestibility = $\frac{\text{amount nitrogen given} - \text{amount nitrogen in feces}}{\text{amount nitrogen given}} \times 100$

was fed 15 percent soy protein for comparison. The rats had an initial weight of 53 g. After 14 days, the mean body weight of the rats fed hair protein was 95 ± 11 g., and of the soy and casein groups 133 ± 11 g. and 162 ± 16 g., respectively. The protein efficiency ratio (PER) is the weight gain divided by the grams of protein intake. The PER values given are the actual values which have been corrected to 2.50 for casein. The PER value of the hair-soy mixture was 1.69, lower than that of soy alone and about two thirds that of the casein control. The nitrogen digestibility of the hair-soy mixture also was lower than that of soy alone and of the casein control. Investigations are being continued to determine whether further treatment could increase the digestibility of the recovered hair proteins.

ACKNOWLEDGMENT

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DISCUSSION

MR. RUSSELL LAKOSKI (Ocean Leather Corp.): As a tanner I would especially like to thank Bill and his host of co-workers for presenting this paper. If you can judge the value of a paper by its co-workers, then this becomes a nine-star production. This type of paper from the Eastern Regional Laboratories is the exact type of paper that a tanner likes to hear, because here they are attacking a real and very immediate problem. I think the meat of this paper comes down to four or five sentences in the summary that Bill made. The recovery of the proteins by the procedure he described will do these three things:

1. It allows for the drastic reduction of the COD and BOD.
2. It allows for the recycling of various sulfiding and liming chemicals.
3. It also produces a very potentially useful product.

Now if we take all three of these and put them together, the ultimate possibly would be a profit from treating your effluent. However, this has to be a long way in the future, perhaps; but if something is done anywhere between that point and paying for the complete treatment of your effluent, then we have made an important gain. With that, I'd like to open up the discussion to questions from the floor.

DR. THOS. C. THORSTENSEN (Thorstensen Laboratory, Westford, Mass.): You mentioned yields. Did you give the number of gallons of effluent that it took to give a pound of protein?

MR. WM. F. HAPPICH (Eastern Marketing and Nutrition Research Div., USDA): Yes. That is right. From the undiluted lime-sulfide effluent from paddle vats, we obtained one pound of protein from approximately 18 to 20 gallons of effluent. This was the undiluted effluent, without any wash water. From the hide processors we were able to obtain one pound of protein from approximately 7 to 9 gallons of undiluted lime-sulfide effluent; this was also without any added wash water.

DR. THORSTENSEN: Would it be possible, if we were to remove the sulfide from a spent lime liquor, to adjust the pH and get a filterable product?

MR. HAPPICH: Do you mean adjusting the pH in the beginning?

DR. THORSTENSEN: No; after we remove the sulfide, by oxidation or some other chemical means, could we then bring the pH to the desired level, kick out the proteins, and then filter?

MR. HAPPICH: Yes, we could. It would be quite possible.

DR. ROSS G. DONOVAN (Canada Packers Ltd., Toronto, Canada): Bill, if I have the figure correct, I believe you said that 32 percent of the COD would be removed by ultra-filtration.

MR. HAPPICH: Yes, by ultra-filtration; that is right.

DR. DONOVAN: How much of that COD would be due to organic, and how much to inorganic materials? Do you have any idea as to this factor?

MR. HAPPICH: No, we have to investigate that part yet. We haven't distinguished between the COD caused by the organic part and the inorganic substances present.

DR. DONOVAN: Thank you. Another question, if I may. Several years ago, we were interested in the cystine content of lime liquors, and we found a very low value. I would like to know how much variability you find in cystine content from process to process.

MR. HAPPICH: So far, all of the proteins we have recovered have been remarkably uniform in composition. There has been very little variation in amino

acid composition, as we demonstrated from the three analyses given on the slides we showed. The variation in composition has been very small.

DR. DONOVAN: Thank you very much.

MRS. JEAN J. TANCOUS (Tanners' Council Laboratory, Cincinnati, Ohio): Is the process with this centrifugation economically feasible? What is the cost of the protein recovery?

MR. HAPPICH: We haven't done a cost analysis yet. We have just begun to do a study of a scale-up of our four-step procedure. We have already started to do some centrifugation studies on some types of commercial centrifuges which could be stepped up to a 20,000 gallons per day system. We have not determined the overall costs as yet.

MRS. TANCOUS: Thank you.

MR. LAKOSKI: In order to stay within the schedule of our time, I would again like to thank Bill Happich and his co-workers for this fine paper presented this morning.
